

BL/CH 401 Lecture #15A -- Introduction to Enzyme Mechanisms

Part I. Features of Enzymatic Catalysis.

Enzymes cause rate increases in reactions that are 10 times to 10,000 times greater than the best chemical catalysts. Enzymes, since they are proteins and must maintain their folded shape or conformation, operate under mild conditions (37°C/pH 7) and have high specificity. Enzymes achieve specificity via geometric and physical complementarity between the active site and the substrate.

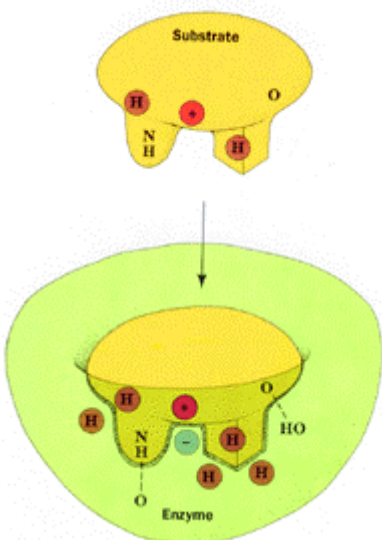


Figure 1. 3-D Geometric Fit of Substrate to Enzyme's Active Site.

Of course, it is somewhat obvious that both the substrate and the enzyme are 3-D and that the shape of the substrate must fit the shape of the active site for the substrate to bind to the enzyme and the E-S complex to form. But an important aspect of this reality is that the enzyme and its active site are both asymmetric in the chemical sense that they are chiral. Amino acids are chiral and so the enzyme and its active site are formed by chiral structures and are themselves chiral. So if the substrate has an degree of chirality (ie either it has a chiral center or even one partially chiral - usually called pro-chiral, then the enzyme will bind the one form or stereo-isomer of the substrate. Let's take the example of an amino acid which is present in both the D- and L-forms, the enzyme will bind only one of these forms, which in most cases will be the L-form of the amino acid.

But how do enzymes achieve enormous rate accelerations? Why are enzymes such good catalysts?

The answer seems to be that enzymes are better designed for catalyzing a reaction than chemical catalysts. On the other hand, a review of chemical catalysis and the concept of a mechanism for reaction is useful for laying the foundation for understanding an enzyme's mechanism of

catalysis.

Part II. Chemical Bond Rearrangement During a Reaction.

One way to understand enzyme catalysis- all chemical reactions occur via bond rearrangements. For example, in ester hydrolysis water splits the ester into acid and alcohol.

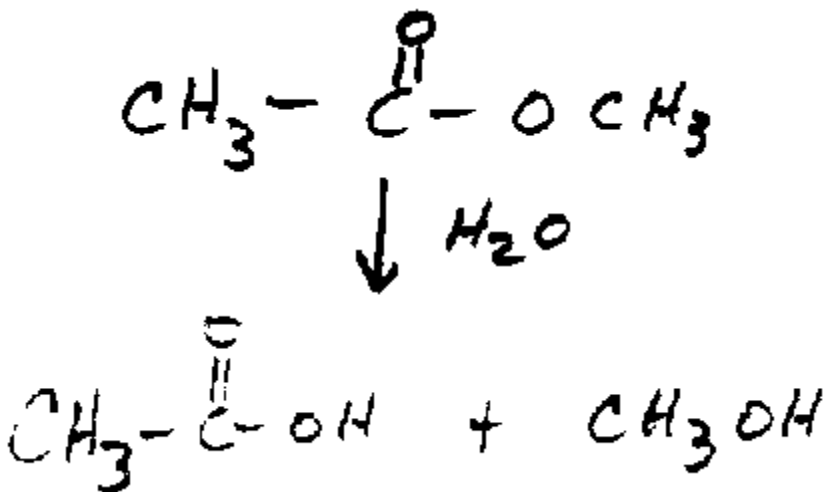


Figure 2. Ester Hydrolysis.

This reaction is slow at neutral pH, but faster at acidic pH. Acid catalyzes ester hydrolysis. But how does H^+ catalyze the reaction? The organic chemist answers this question by hypothesizing a 'mechanism' for the reaction, which explains how acid increases bond rearrangement. First, a hypothesis for the mechanism of the reaction in the absence of a catalyst. This mechanism is a plausible idea for how the bonds rearrange during the reaction between the ester and water. Thus, the mechanism of the reaction illustrates a likely course for the bond rearrangements to take during the reaction.

Figure 3. Mechanism of Ester Hydrolysis in Absence of Catalyst.

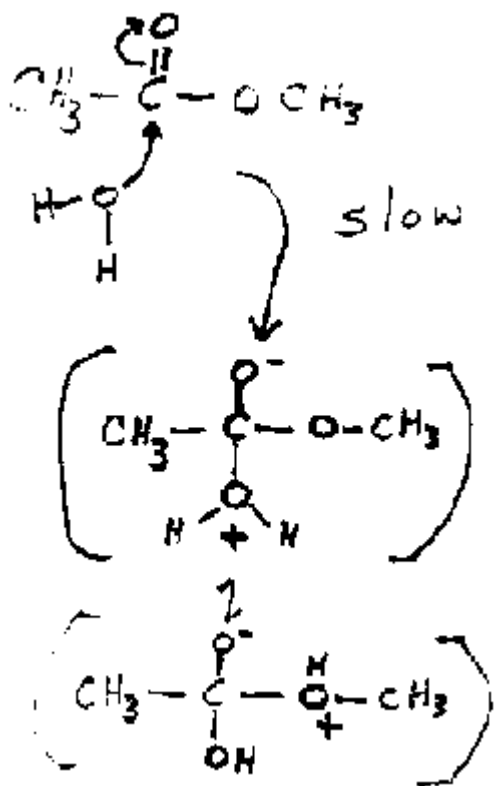


Figure 3A. Attack of the Water Molecule on the Ester.

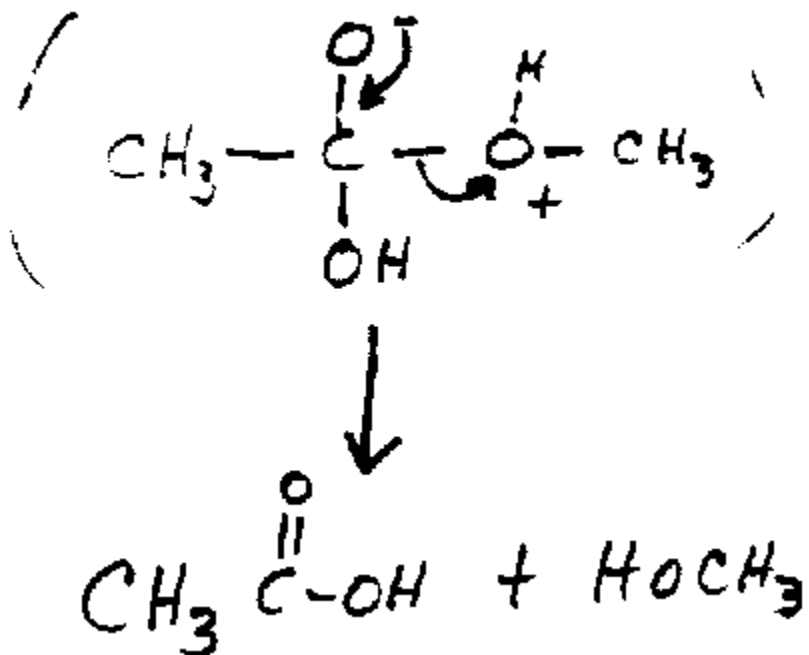


Figure 3B. Decomposition of the Ester-Water Intermediate into Acid and Alcohol.

The molecules shown in brackets are hypothesized intermediates in the reaction, which are very difficult to actually demonstrate by chemical analysis such as spectroscopy or NMR (nuclear

magnetic resonance). The actual bond rearrangements take place in the intermediates in the reaction, which are shown by arrows illustrating the movement of electron pairs to form the new bonds of the products. In this case, the slowest step is the attack of the water molecule on the ester, which is illustrated by the 'free' electrons of the oxygen in water attacking an electron-deficient center in the ester (Fig. 3A). It has become known that the most electron deficient atom in an ester is the carbonyl's carbon. Thus, the hypothesis is developed based on this idea and water attack on the ester leads to plausible intermediates based on bond rearrangements which logically follow from the first step in the reaction, which is the slowest or rate-determining step. Then the intermediates rapidly rearrange bonds resulting in a good suggestion for how the ester-water complex breaks down to form the products, which happens very fast after the slow reaction where the water attacks the ester is done.

Part III. Acid Catalyzed Ester Hydrolysis.

Now, acid catalysis of ester hydrolysis can be hypothesized in a slightly altered mechanism, which shows how the catalyst changes the course of bond rearrangements. It is clear at the start that the acid catalyst must accelerate the first step in the reaction, ie the attack of the water molecule on the ester.

Acid Catalyzed Ester Hydrolysis:

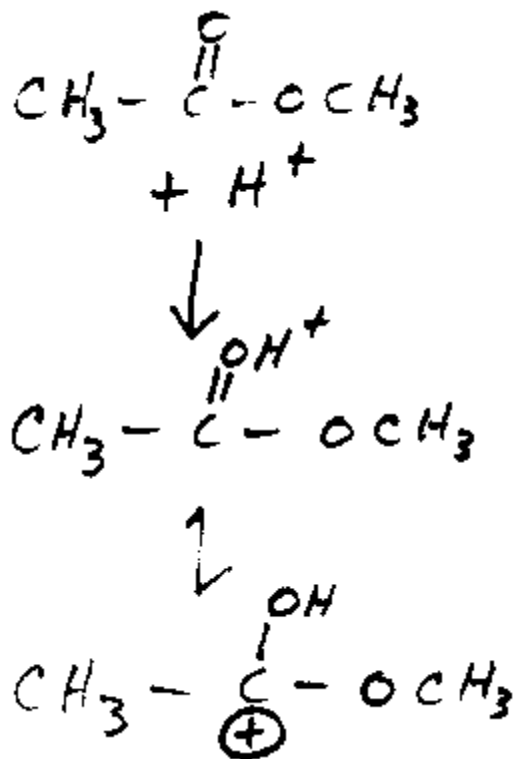


Figure 4. Acid Activation of the Ester.

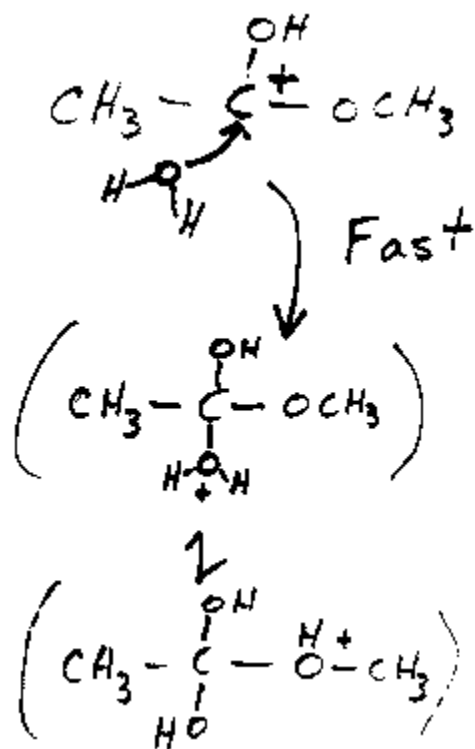


Figure 5. Attack of Water and the Resulting Intermediates.

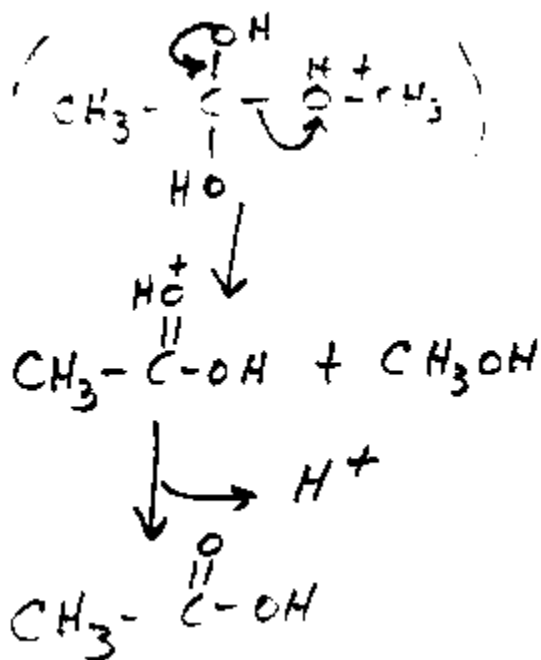


Figure 6. Decomposition of Ester-Acid-Water Intermediates to Products.

The final step in the process is the regeneration of the acid catalysts (ie the proton is released from organic "acid").

SUMMARY

All the steps in the hypothesized mechanism must fit with known properties of the chemical involved in the mechanism and if possible, chemical evidence for the various bond rearrangements in the mechanism should be obtained. This simple example of a mechanism of reaction from organic chemistry illustrates what a biochemist must do in order to explain what happens when an enzyme catalyzes a similar reaction such as trypsin catalyzing the hydrolysis of a protein's backbone. In this case, water attacks an amide bond, the peptide bond, which is very similar to the ester hydrolysis illustrated above. Thus, the question is how do the amino acid side chains of an enzyme assist in bond rearrangements? Can a plausible mechanism of action for enzyme catalyzed peptide bond hydrolysis be formulated by the biochemist in a manner similar to that done by the organic chemist? These questions are addressed in the next lecture.

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